

about 10-30, preferably about 16-24, injection sites per animal. One  $\mu$ l of Evans blue dye (1 % in physiologic buffered saline) is injected intracardially. Blemishes at the injection sites are then measured (mm diameter) at 1 hr and 6 hr post injection. Animals were sacrificed at 6 hrs after injection. Each skin injection site is biopsied and fixed in formalin. The skins are then prepared for histopathologic evaluation. Each site is evaluated for inflammatory cell infiltration into the skin. Sites with visible inflammatory cell inflammation are scored as positive. Inflammatory cells may be neutrophilic, eosinophilic, monocytic or lymphocytic. At least a minimal perivascular infiltrate at the injection site is scored as positive, no infiltrate at the site of injection is scored as negative.

The following polypeptide tested positive in this assay: PRO1283, PRO1325 and PRO1343.

#### 10 EXAMPLE 142: Induction of c-fos in Endothelial Cells (Assay 34)

This assay is designed to determine whether PRO polypeptides show the ability to induce c-fos in endothelial cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of conditions or disorders where angiogenesis would be beneficial including, for example, wound healing, and the like (as would agonists of these PRO polypeptides). Antagonists of the PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of cancerous tumors.

Human venous umbilical vein endothelial cells (HUVEC, Cell Systems) in growth media (50% Ham's F12 w/o GHT: low glucose, and 50% DMEM without glycine: with  $\text{NaHCO}_3$ , 1% glutamine, 10 mM HEPES, 10% FBS, 10 ng/ml bFGF) were plated on 96-well microtiter plates at a cell density of  $1 \times 10^4$  cells/well. The day after plating, the cells were starved by removing the growth media and treating the cells with 100  $\mu$ l/well test samples and controls (positive control = growth media; negative control = Protein 32 buffer = 10 mM HEPES, 140 mM NaCl, 4% (w/v) mannitol, pH 6.8). The cells were incubated for 30 minutes at 37°C, in 5%  $\text{CO}_2$ . The samples were removed, and the first part of the bDNA kit protocol (Chiron Diagnostics, cat. #6005-037) was followed, where each capitalized reagent/buffer listed below was available from the kit.

Briefly, the amounts of the TM Lysis Buffer and Probes needed for the tests were calculated based on information provided by the manufacturer. The appropriate amounts of thawed Probes were added to the TM Lysis Buffer. The Capture Hybridization Buffer was warmed to room temperature. The bDNA strips were set up in the metal strip holders, and 100  $\mu$ l of Capture Hybridization Buffer was added to each b-DNA well needed, followed by incubation for at least 30 minutes. The test plates with the cells were removed from the incubator, and the media was gently removed using the vacuum manifold. 100  $\mu$ l of Lysis Hybridization Buffer with Probes were quickly pipetted into each well of the microtiter plates. The plates were then incubated at 55°C for 15 minutes. Upon removal from the incubator, the plates were placed on the vortex mixer with the microtiter adapter head and vortexed on the #2 setting for one minute. 80  $\mu$ l of the lysate was removed and added to the bDNA wells containing the Capture Hybridization Buffer, and pipetted up and down to mix. The plates were incubated at 53°C for at least 16 hours.

On the next day, the second part of the bDNA kit protocol was followed. Specifically, the plates were removed from the incubator and placed on the bench to cool for 10 minutes. The volumes of additions needed were calculated based upon information provided by the manufacturer. An Amplifier Working Solution was

prepared by making a 1:100 dilution of the Amplifier Concentrate (20 fm/ $\mu$ l) in AL Hybridization Buffer. The hybridization mixture was removed from the plates and washed twice with Wash A. 50  $\mu$ l of Amplifier Working Solution was added to each well and the wells were incubated at 53°C for 30 minutes. The plates were then removed from the incubator and allowed to cool for 10 minutes. The Label Probe Working Solution was prepared by making a 1:100 dilution of Label Concentrate (40 pmoles/ $\mu$ l) in AL Hybridization Buffer. After the 10-minute cool-down period, the amplifier hybridization mixture was removed and the plates were washed twice with Wash A. 50  $\mu$ l of Label Probe Working Solution was added to each well and the wells were incubated at 53°C for 15 minutes. After cooling for 10 minutes, the Substrate was warmed to room temperature. Upon addition of 3  $\mu$ l of Substrate Enhancer to each ml of Substrate needed for the assay, the plates were allowed to cool for 10 minutes, the label hybridization mixture was removed, and the plates were washed twice with Wash A and three times with Wash D. 50  $\mu$ l of the Substrate Solution with Enhancer was added to each well. The plates were incubated for 30 minutes at 37°C and RLU was read in an appropriate luminometer.

The replicates were averaged and the coefficient of variation was determined. The measure of activity of the fold increase over the negative control (Protein 32/HEPES buffer described above) value was indicated by chemiluminescence units (RLU). The results are considered positive if the PRO polypeptide exhibits at least a two-fold value over the negative buffer control. Negative control = 1.00 RLU at 1.00% dilution. Positive control = 8.39 RLU at 1.00% dilution.

The following PRO polypeptides tested positive in this assay: PRO1274, PRO1294, PRO1304 and PRO1130.

#### 20 EXAMPLE 143: Gene Amplification

This example shows that the PRO1295-, PRO1293-, PRO1265-, PRO1303-, PRO1269-, PRO1410-, PRO1317-, PRO1780-, PRO1555-, PRO1755-, PRO1558-, PRO1759- and PRO1788-encoding genes are amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the polypeptides are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast and other cancers. Therapeutic agents may take the form of antagonists of PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 polypeptides, for example, murine-human chimeric, humanized or human antibodies against a PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 or PRO1788 polypeptide.

The starting material for the screen was genomic DNA isolated from a variety of cancers. The DNA is quantitated precisely, *e.g.*, fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy individuals (not shown). The 5' nuclease assay (for example, TaqMan™) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System™ (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNA encoding PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 7. An explanation of the abbreviations used

for the designation of the primary tumors listed in Table 7 and the primary tumors and cell lines referred to throughout this example has been given hereinbefore.

5 The results of the TaqMan™ are reported in delta ( $\Delta$ ) Ct units. One unit corresponds to 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a TaqMan™ fluorescent probe derived from the PRO1295-, PRO1293-, PRO1265-, PRO1303-, PRO1269-, PRO1410-, PRO1317-, PRO1780-, PRO1555-, PRO1755-, PRO1558-, PRO1759- and PRO1788-encoding gene. Regions of PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, *e.g.*, 3'-untranslated regions. The  
10 sequences for the primers and probes (forward, reverse and probe) used for the PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 gene amplification analysis were as follows:

PRO1295 (DNA59218-1559)

forward: 5'-AGGACTTGCCCTCAGGAA-3' (SEQ ID NO:432)

reverse: 5'-CGCAGGACAGTTGTGAAAATA-3' (SEQ ID NO:433)

probe: 5'-ATGACGCTCGTCCAAGGCCAC-3' (SEQ ID NO:434)

5 PRO1293 (DNA60618-1557)

forward: 5'-CCCACCTGTACCACCATGT-3' (SEQ ID NO:435)

probe: 5'-ACTCCAGGCACCATCTGTTCTCCC-3' (SEQ ID NO:436)

reverse: 5'-AAGGGCTGGCATTCAAGTU-3' (SEQ ID NO:437)

10 PRO1265 (DNA60764-1533)

forward: 5'-TGACCTGGCAAAGGAAGAA-3' (SEQ ID NO:438)

probe: 5'-CAGCCACCCTCCAGTCCAAGG-3' (SEQ ID NO:439)

reverse: 5'-GGGTCGTGTTTTGGAGAGA-3' (SEQ ID NO:440)

15 PRO1303 (DNA65409-1566)

forward: 5'-CTGGCCCTCAGAGCACCAAT-3' (SEQ ID NO:441)

probe: 5'-TCCTCCATCACTTCCCCTAGCTCCA-3' (SEQ ID NO:442)

reverse: 5'-CTGGCAGGAGTTAAAGTTCCAAGA-3' (SEQ ID NO:443)

20 PRO1269 (DNA66520-1536)

forward: 5'-AAAGGACACCGGGATGTG-3' (SEQ ID NO:444)

probe: 5'-AGCGTACACTCTCTCCAGGCAACCAG-3' (SEQ ID NO:445)

reverse: 5'-CAATTCTGGATGAGGTGGTAGA-3' (SEQ ID NO:446)

25 PRO1410 (DNA68874-1622)

forward: 5'-CAGGACTGAGCGCTTGTTTA-3' (SEQ ID NO:447)

probe: 5'-CAAAGCGCCAAGTACCGGACC-3' (SEQ ID NO:448)

reverse: 5'-CCAGACCTCAGCCAGGAA-3' (SEQ ID NO:449)

30 PRO1317 (DNA71166-1685)

forward: 5'-CCCTAGCTGACCCCTTCA-3' (SEQ ID NO:450)

reverse: 5'-TCTGACAAGCAGTTTTCTGAATC-3' (SEQ ID NO:451)

probe: 5'-CTCTCCCCCTCCCTTTTCTTTGTTT-3' (SEQ ID NO:452)

35 PRO1780 (DNA71169-1709)

forward: 5'-CTCTGGTGCCACAGTGA-3' (SEQ ID NO:453)

probe: 5'-CCATGCCTGCTCAGCCAAGAA-3' (SEQ ID NO:454)

reverse: 5'-CAGGAAATCTGGAAACCTACAGT-3' (SEQ ID NO:455)

PRO1555 (DNA73744-1665)

forward: 5'-CCTTGAAAAGGACCCAGTTT-3' (SEQ ID NO:456)

probe: 5'-ATGAGTCGCACCTGCTGTTCCC-3' (SEQ ID NO:457)

reverse: 5'-TAGCAGCTGCCCTTGGTA-3' (SEQ ID NO:458)

forward: 5'-AACAGCAGGTGCGACTCATCTA-3' (SEQ ID NO:459)

5 probe: 5'-TGCTAGGCGACGACACCCAGACC-3' (SEQ ID NO:460)

reverse: 5'-TGGACACGTGGCAGTGGA-3' (SEQ ID NO:461)

PRO1755 (DNA76396-1698)

forward: 5'-TCATGGTCTCGTCCCATTTC-3' (SEQ ID NO:462)

10 probe: 5'-CACCATTTGTTTCTCTGTCTCCCCATC-3' (SEQ ID NO:463)

reverse: 5'-CCGGCATCCTTGGAGTAG-3' (SEQ ID NO:464)

PRO1788 (DNA77652-2505)

forward: 5'-TCCCCATTAGCACAGGAGTA-3' (SEQ ID NO:465)

15 probe: 5'-AGGCTCTTGCCTGTCTGCTGCT-3' (SEQ ID NO:466)

reverse: 5'-GCCCAGAGTCCCACTTGT-3' (SEQ ID NO:467)

PRO1558 (DNA71282-1668)

forward: 5'-ACTGCTCCGCCTACTACGA -3' (SEQ ID NO:468)

20 probe: 5'-AGGCATCCTCGCCGTCCTCA -3' (SEQ ID NO:469)

reverse: 5'-AAGGCCAAGGTGAGTCCAT -3' (SEQ ID NO:470)

forward: 5'-CGAGTGTGTGCGAAACCTAA -3' (SEQ ID NO:471)

probe: 5'-TCAGGGTCTACATCAGCCTCCTGC -3' (SEQ ID NO:472)

reverse: 5'-AAGGCCAAGGTGAGTCCAT -3' (SEQ ID NO:473)

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PRO1759 (DNA76531-1701)

forward: 5'-CCTACTGAGGAGCCCTATGC -3' (SEQ ID NO:474)

probe: 5'-CCTGAGCTGTAACCCCACTCCAGG -3' (SEQ ID NO:231)

reverse: 5'-AGAGTCTGTCCCAGCTATCTTGT -3' (SEQ ID NO:232)

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The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore.

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One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

5 The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI Prism 7700™ Sequence Detection. The system consists of a thermocycler, laser, charge-coupled device (CCD) camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

10 5' Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The  $\Delta C_t$  values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

Table 7 describes the stage, T stage and N stage of various primary tumors which were used to screen the PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 compounds of the invention.

Table 7  
Primary Lung and Colon Tumor Profiles

	Primary Tumor	Stage	Other Stage	Dukes Stage	T Stage	N Stage
	Human lung tumor AdenoCa (SRCC724) [LT1]	IIA			T1	N1
5	Human lung tumor SqCCa (SRCC725) [LT1a]	IIB			T3	N0
	Human lung tumor AdenoCa (SRCC726) [LT2]	IB			T2	N0
	Human lung tumor AdenoCa (SRCC727) [LT3]	IIIA			T1	N2
	Human lung tumor AdenoCa (SRCC728) [LT4]	IB			T2	N0
	Human lung tumor SqCCa (SRCC729) [LT6]	IB			T2	N0
10	Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IA			T1	N0
	Human lung tumor AdenoCa (SRCC731) [LT9]	IB			T2	N0
	Human lung tumor SqCCa (SRCC732) [LT10]	IIB			T2	N1
	Human lung tumor SqCCa (SRCC733) [LT11]	IIA			T1	N1
	Human lung tumor AdenoCa (SRCC734) [LT12]	IV			T2	N0
15	Human lung tumor AdenoSqCCa (SRCC735) [LT13]	IB			T2	N0
	Human lung tumor SqCCa (SRCC736) [LT15]	IB			T2	N0
	Human lung tumor SqCCa (SRCC737) [LT16]	IB			T2	N0
	Human lung tumor SqCCa (SRCC738) [LT17]	IIB			T2	N1
	Human lung tumor SqCCa (SRCC739) [LT18]	IB			T2	N0
20	Human lung tumor SqCCa (SRCC740) [LT19]	IB			T2	N0
	Human lung tumor LCCa (SRCC741) [LT21]	IIB			T3	N1
	Human lung AdenoCa (SRCC811) [LT22]	IA			T1	N0
	Human colon AdenoCa (SRCC742) [CT2]		M1	D	pT4	N0
	Human colon AdenoCa (SRCC743) [CT3]			B	pT3	N0
25	Human colon AdenoCa (SRCC 744) [CT8]			B	T3	N0
	Human colon AdenoCa (SRCC745) [CT10]		A	pT2	N0	
	Human colon AdenoCa (SRCC746) [CT12]	MO, R1	B	T3	N0	
	Human colon AdenoCa (SRCC747) [CT14]	pMO, RO	B	pT3	pN0	
	Human colon AdenoCa (SRCC748) [CT15]	M1, R2	D	T4	N2	
30	Human colon AdenoCa (SRCC749) [CT16]	pMO	B	pT3	pN0	
	Human colon AdenoCa (SRCC750) [CT17]		C1	pT3	pN1	
	Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	N0
	Human colon AdenoCa (SRCC752) [CT4]			B	pT3	M0
	Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pN0
35	Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pN0
	Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pN0
	Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
	Human colon AdenoCa (SRCC757) [CT11]		B	T3	N0	
40	Human colon AdenoCa (SRCC758) [CT18]	MO, RO	B	pT3	pN0	

#### DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, and normal human blood. The isolation was performed using purification kit, buffer set and protease and all from Qiagen, according to the manufacturer's instructions and the description below.

#### 45 Cell culture lysis:

Cells were washed and trypsinized at a concentration of  $7.5 \times 10^8$  per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS and recentrifugation. The pellets were washed a third time, the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 ml PBS. Buffer C1 was equilibrated at 4°C. Qiagen protease #19155 was diluted into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 ml of G2 Buffer was prepared by diluting Qiagen RNase A stock (100 mg/ml) to a final concentration of 200 µg/ml.

Buffer C1 (10 ml, 4°C) and ddH<sub>2</sub>O (40 ml, 4°C) were then added to the 10 ml of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a Beckman swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the nuclei were suspended with a vortex into 2 ml Buffer C1 (at 4°C) and 6 ml ddH<sub>2</sub>O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200  $\mu$ l per tip. 5 G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Quiagen protease (200  $\mu$ l, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Solid human tumor sample preparation and lysis:*

10 Tumor samples were weighed and placed into 50 ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNase A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenated in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar- 15 flow TC hood in order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH<sub>2</sub>O, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Quiagen protease (prepared as indicated above, 1.0 ml) was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation were repeated until the lysates were clear (e.g., 20 incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Human blood preparation and lysis:*

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Quiagen protease was freshly prepared by dilution into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNase A to a final 25 concentration of 200  $\mu$ g/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50 ml conical tube and 10 ml C1 buffer and 30 ml ddH<sub>2</sub>O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a Beckman swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH<sub>2</sub>O (4°C). Vortexing was repeated until the pellet was white. The nuclei were 30 then suspended into the residual buffer using a 200  $\mu$ l tip. G2 buffer (10 ml) was added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Quiagen protease was added (200  $\mu$ l) and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Purification of cleared lysates:*

35 (1) Isolation of genomic DNA:

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30 ml silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each



sample, the tubes covered with parafin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

(2) Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard  $A_{260}/A_{280}$  spectrophotometry on a 1:20 dilution (5  $\mu$ l DNA + 95  $\mu$ l ddH<sub>2</sub>O) using the 0.1 ml quartz cuvettes in the Beckman DU640 spectrophotometer.  $A_{260}/A_{280}$  ratios were in the range of 1.8-1.9. Each DNA sample was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/ $\mu$ l), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10  $\mu$ l, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2 ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2  $\mu$ l, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2  $\mu$ l of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometrically determined concentration was then used to dilute each sample to 10 ng/ $\mu$ l in ddH<sub>2</sub>O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman<sup>TM</sup> primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was +/- 1 Ct. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough for 8-9 plates or 64 tests.

Gene amplification assay:

The PRO1295, PRO1293, PRO1265, PRO1303, PRO1269, PRO1410, PRO1317, PRO1780, PRO1555, PRO1755, PRO1558, PRO1759 and PRO1788 compounds of the invention were screened in the following primary tumors and the resulting  $\Delta$ Ct values which are  $\geq 1.0$  are reported in Table 8.

Table 8

 $\Delta$ Ct values in lung and colon primary tumors and cell line models

	Primary Tumors or Cell lines	PRO 1293	PRO 1269	PRO 1410	PRO 1755	PRO 1780	PRO 1788	PRO 1303	PRO 1555	PRO 1265	PRO 1317	PRO 1295	PRO 1558	PRO 1759
5	LT1	--	--	--	--	--	--	--	--	--	1.15	--	--	--
	LT1-a	--	--	--	--	--	--	--	--	--	1.49	--	--	--
10	LT3	--	--	--	--	--	--	--	--	1.04	--	--	--	--
	LT4	--	--	--	--	1.16	--	--	--	--	--	--	--	--
	LT7	--	--	--	--	1.02	--	--	--	--	--	--	--	--
	LT9	--	--	--	--	--	--	--	--	--	1.26	--	--	--
	LT10	--	--	--	--	--	--	--	--	--	1.68	--	--	--
15	LT12	--	--	--	--	--	--	--	--	2.17	--	--	--	--
	LT13	--	--	1.12 1.42	--	--	--	1.42	4.20 4.45	2.24	--	--	--	--
	LT15	--	1.22	2.10 1.82	--	--	--	1.17	1.36 1.15	3.51	1.16	--	--	--
	LT16	--	1.14	1.44 1.45	1.36	--	--	1.42	3.71 3.99	3.32	--	--	--	--
	LT17	--	1.26	--	--	--	--	--	--	1.02	1.74	--	--	--
20	LT18	--	--	--	1.18	--	--	--	--	--	--	--	--	--
	CT2	--	--	2.36	2.35	--	--	--	--	--	--	--	--	--
	CT3	--	--	1.09	--	--	1.35	--	--	--	--	--	--	--
	CT8	--	--	--	1.64	--	1.26	--	--	--	--	--	--	--
	CT10	--	--	1.41	2.05	--	1.37	--	--	--	--	--	--	--
25	CT12	--	--	--	1.15	--	1.24	--	--	--	--	--	--	--
	CT14	--	--	1.46	1.40	--	2.58	--	--	--	--	--	--	--
	CT15	--	--	--	--	--	--	--	1.34 1.62	--	--	--	--	--
	CT16	--	--	--	--	--	--	1.13	1.04 1.05	--	--	--	--	--
	CT17	--	--	--	--	--	--	--	1.16	--	--	--	--	--
30	CT1	--	--	--	--	--	1.09	--	--	--	--	--	--	--
	CT4	--	--	--	--	--	1.22	--	--	--	--	--	--	--
	CT5	--	--	2.14	--	--	--	--	--	--	--	--	--	--
	CT9	--	--	--	--	--	1.52	--	--	--	--	--	--	--
	CT11	--	--	1.29	--	--	--	--	--	--	--	--	--	--
35	A549	--	--	--	--	--	--	1.20	2.17 2.11	--	--	--	--	--

5	Calu-1	--	--	--	--	--	--	1.39	--	--	--	--	--
	Calu-6	--	--	--	--	--	--	1.12	--	--	--	--	--
	H441	--	--	--	--	--	--	2.06	--	--	--	--	--
	H460	--	--	--	--	--	--	1.88	--	--	--	--	--
	SKMES 1	--	--	--	--	--	--	1.90	--	--	--	--	--
	SW620	--	--	--	--	--	--	2.24	--	--	--	--	--
	Colo320	--	--	--	--	--	--	2.21 2.24	--	--	--	--	--
	HT29	--	--	1.22	--	--	--	--	--	--	--	--	--
	HCT116	--	--	--	--	--	--	2.46 2.66	--	--	--	--	--
	LT22	--	--	--	1.26	1.07	--	--	--	2.69	--	--	--
10	HF- 000716	--	--	--	--	--	--	2.63 2.73	--	--	--	--	--
	HF- 000733	--	--	--	--	--	--	2.58 2.71 1.39	--	--	--	--	--
	HF- 000611	--	--	--	--	--	--	4.99	--	--	--	--	--
	HF- 000539	2.33	--	--	--	--	--	3.13 2.55	--	--	1.49	--	--
	HF- 000575	--	--	--	--	--	--	1.32	--	--	--	--	--
	HF- 000698	--	--	--	--	--	--	--	--	--	1.09	--	--
	HF- 000545	--	--	--	--	--	--	1.59 1.68	--	--	1.11	--	--
	HF- 000631	--	--	--	--	--	--	1.37	--	--	1.27	--	--
	HF- 000840	1.71	--	--	--	--	--	3.63	--	--	1.97	1.39	1.11
	HF- 000842	--	--	--	--	--	--	1.99	--	--	--	1.24	--
15	HF- 000795	1.13	--	--	--	--	--	--	--	--	--	1.01	1.32
	HF- 001294	--	--	--	--	--	--	--	--	--	--	1.50	--
	HF- 001296	--	--	--	--	--	--	--	--	--	--	2.88	1.51
	HF- 001299	--	--	--	--	--	--	--	--	--	--	1.37	--

### PRO1265

PRO1265 (DNA60764-1533) was also reexamined along with selected tumors from the above initial screen with framework mapping. Table 9 indicates the chromosomal mapping of the framework markers that were used in the present example. The framework markers are located approximately every 20 megabases and were used to control aneuploidy.

PRO1265 was also reexamined with epicenter mapping. The markers indicated in Table 10 are located in close proximity (in the genome) to DNA60764-1533, and are used to assess the relative amplification in the immediate vicinity of Chromosome 19 wherein the molecule is located. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-33698 is closest to DNA60764-1533.

Table 9  
Framework Markers Along Chromosome 19

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name
S12	AFMa107xc9
S50	SHGC-31335
S105	SHGC-34102
S155	SHGC-16175

Table 10  
Epicenter Markers Along Chromosome 19 used for DNA60764-1533

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
DNA34353	—	maps to S158
DNA40620	—	maps to S160
DNA54002	—	maps to S160
S160	SHGC-34723	21
DNA60764	—	—
S161	SHGC-30929	15
S162	SHGC-10328	17
S163	AFMa115wg5	—

The  $\Delta C_t$  values of the above described framework markers along Chromosome 19 relative to PRO1265 are indicated for selected tumors in Table 11.

Table 11

Amplification of framework markers relative to DNA60764-1533 ( $\Delta C_t$ )

Tumor	Framework Markers				
	S12	DNA60764-1533	S50	S105	S155
LT1	0.16	0.06	-0.42	0.11	-1.56
LT1a	0.05	-0.27	0.17	0.40	0.00
LT2	0.48	0.41	0.52	0.13	-0.36
LT3	0.27	0.83	0.11	0.50	1.04
LT4	0.48	0.67	0.20	0.56	-0.35
LT6	0.72	0.74	0.32	0.35	0.24
LT7	0.82	0.85	0.95	0.95	0.75
LT9	0.72	0.61	0.19	0.64	-0.35
LT10	0.82	0.98	0.62	0.53	0.32
LT11	0.13	0.25	0.55	-0.34	0.70
LT12	0.04	0.60	0.21	-0.17	2.17
LT13	-0.06	0.57	-0.30	-0.05	2.24
LT15	-0.03	-0.77	0.12	-0.04	3.51
LT16	0.46	1.37	0.51	0.23	3.32
LT17	0.37	0.74	0.21	0.22	1.02
LT18	0.39	0.57	0.11	0.16	0.52
LT22	0.79	0.76	-0.05	0.16	0.59

DISCUSSION AND CONCLUSION:PRO1269 (DNA66520-1536):

The  $\Delta C_t$  values for DNA66520-1536 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA66520-1536 encoding PRO1269 occurred in primary lung tumors: LT15, LT16 and LT17. Because amplification of DNA66520-1536 occurs in various lung tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA66520-1536 (PRO1269) would be expected to have utility in cancer therapy.

PRO1410 (DNA68874-1622):

The  $\Delta C_t$  values for DNA68874-1622 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. the

above data indicates that significant amplification of nucleic acid DNA68874-1622 encoding PRO1410 occurred: (1) in primary lung tumors: LT13, LT15 and LT16; (2) in primary colon tumors: CT2, CT3, CT5, CT10, CT11, and CT14; and (3) in colon cell line HT29. Because amplification of DNA68874-1622 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA68874-1622 (PRO1410) would be expected to have utility in cancer therapy.

PRO1755 (DNA76396-1698):

The  $\Delta C_t$  values for DNA76396-1698 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA76396-1698 encoding PRO1755 occurred: (1) in primary lung tumors: LT16, LT18 and LT22; and (2) in primary colon tumors: CT2, CT8, CT10, CT12, and CT14. Because amplification of DNA76396-1698 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA76396-1698 (PRO1755) would be expected to have utility in cancer therapy.

PRO1780 (DNA71169-1709):

The  $\Delta C_t$  values for DNA71169-1709 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA71169-1709 encoding PRO1780 occurred in primary lung tumors: LT4, LT7 and LT22. Because amplification of DNA71169-1709 occurs in various lung tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA71169-1709 (PRO1780) would be expected to have utility in cancer therapy.

PRO1788 (DNA77652-2505):

The  $\Delta C_t$  values for DNA77652-2505 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA77652-2505 encoding PRO1788 occurred in primary colon tumors: CT1, CT3, CT4, CT8, CT9, CT10, CT12, and CT14. Because amplification of DNA77652-2505 occurs in various colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA77652-2505 (PRO1788) would be expected to have utility in cancer therapy.

PRO1295 (DNA59218-1559):

The  $\Delta C_t$  values for DNA59218-1559 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA59218-1559 encoding PRO1295 occurred: (1) in primary lung tumors: HF-000631 and HF-000840; (2) colon tumor centers: HF-000539 and HF-000698;

and (3) in breast tumor center HF-000545. Because amplification of DNA59218-1559 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA59218-1559 (PRO1295) would be expected to have utility in cancer therapy.

5 PRO1293 (DNA60618-1557):

The  $\Delta C_t$  values for DNA60618-1557 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA60618-1557 encoding PRO1293 occurred: (1) in primary lung tumor HF-000840; and (2) in colon tumor centers: HF-000539 and HF-000795. Because amplification of DNA60618-1557 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA60618-1557 (PRO1293) would be expected to have utility in cancer therapy.

15 PRO1303 (DNA65409-1566):

The  $\Delta C_t$  values for DNA65409-1566 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA65409-1566 encoding PRO1303 occurred: (1) in primary lung tumors: LT13, LT15 and LT16; (2) in lung cell line A549; and (3) in colon tumor CT16. Because amplification of DNA65409-1566 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA65409-1566 (PRO1566) would be expected to have utility in cancer therapy.

20 PRO1555 (DNA73744-1665):

The  $\Delta C_t$  values for DNA73744-1665 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA73744-1665 encoding PRO1555 occurred: (1) in primary lung tumors: LT13, LT15, LT16, HF-000631, HF-000840, and HF-000842; (2) in lung cell lines: A549, Calu-1, Calu-6, H441, H460, and SKMES1; (3) in primary colon tumors: CT15, CT16, CT17, and colon tumor centers HF-000539 and HF-000575; (4) in colon cell lines: SW620, Colo320 and HCT116; (5) in breast tumor center HF-000545; (6) in kidney tumor center HF-000611; and (7) in testis tumor margin HF-000716 and testis tumor center HF-000733. Because amplification of DNA73744-1665 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA73744-1665 (PRO1555) would be expected to have utility in cancer therapy.

35 PRO1265 (DNA60764-1533):

The  $\Delta C_t$  values for DNA60764-1533 in a variety of lung tumors are reported above. A  $\Delta C_t$  value of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of DNA60765-1533 occurred in primary lung

tumors LT3, LT12, LT13, LT15, LT16 and LT17. The  $\Delta C_t$  values of these hits are 1.03, 2.17, 2.24, 3.51, 3.32 and 1.02. This represents an increase in gene copy of approximately 2.04, 4.50, 4.72, 11.39, 9.99 and 2.03.

Amplification has also been confirmed framework mapping for DNA60764-1533 in LT16. The reported  $\Delta C_t$  value was 1.37, which represents a 2.58 fold increase in gene copy relative to normal tissue. Epicenter mapping has also confirmed amplification of DNA60764-1533 in LT12, LT13, LT15, LT16, CT1, CT4, CT5, CT7 and CT11. These tumors report  $\Delta C_t$  values of 2.35, 2.37, 3.88, 3.32 in the lung tumors and 1.74, 1.86, 3.28, 1.29 and 2.32 in the colon tumors. Relative to normal tissue, this represents an increase in gene copy of approximately 5.10, 5.17, 14.72 and 9.98 in the lung tumors and 3.34, 3.63, 9.71, 2.45 and 4.99 in the colon tumors.

In contrast, the amplification of the closest known framework markers, epicenter markers and the comparison sequences does not occur to a greater extent than that of DNA60764-1533. This strongly suggests that DNA60764-1533 is the gene responsible for the amplification of the particular region in Chromosome 19. Because amplification of DNA60764-1533 occurs in various lung and colon tumors, it is highly probably to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA60764-1533 would be expected to have utility in cancer therapy.

#### PRO1317 (DNA71166-1685):

The  $\Delta C_t$  values for DNA71166-1685 in a variety of tumors are reported above. A  $\Delta C_t$  of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA71166-1685 encoding PRO1317 occurred in primary lung tumors LT1, LT1a, LT9, LT10, LT15, LT17 and LT22. Because amplification of DNA71166-1685 occurs in various tumors, it is likely associated with tumor formation and/or growth. As a result, antagonists (*e.g.*, antibodies) directed against PRO1317 would be expected to be useful in cancer therapy.

#### Summary

Because amplification of the various DNA's as described above occurs in various tumors, they are likely associated with tumor formation and/or growth. As a result, antagonists (*e.g.*, antibodies) directed against these polypeptides would be expected to be useful in cancer therapy.

#### EXAMPLE 144: Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (Assay 24)

This example shows that certain polypeptides of the invention are active as a stimulator of the proliferation of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.



More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 3x10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50 :1 of irradiated stimulator cells, and

50 :1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO<sub>2</sub> for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 30 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x10<sup>6</sup> cells/ml of assay media. The assay is then conducted as described above.

Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

The following PRO polypeptides tested positive in this assay: PRO1246 and PRO1343.

#### EXAMPLE 145: Mouse Kidney Mesangial Cell Proliferation Assay (Assay 92)

This assay shows that certain polypeptides of the invention act to induce proliferation of mammalian kidney mesangial cells and, therefore, are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with Schönlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease. The assay is performed as follows. On day one, mouse kidney mesangial cells are plated on a 96 well plate in growth media (3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, PRO polypeptides are diluted at 2 concentrations (1% and 0.1%) in serum-free medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20μl of the Cell Titer 96 Aqueous one solution reagent (Progema) was added to each well and the colorimetric reaction was allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is anything that gives an absorbance reading which is at least 15% above the control reading.

The following polypeptide tested positive in this assay: PRO1265, PRO1244 and PRO1382.

#### EXAMPLE 146: Induction of Pancreatic β-Cell Precursor Differentiation (Assay 89)

This assay shows that certain polypeptides of the invention act to induce differentiation of pancreatic

$\beta$ -cell precursor cells into mature pancreatic  $\beta$ -cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either  $\beta$ -cell precursors or mature  $\beta$ -cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is insulin.

5 The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20 $\mu$ g/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary culture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant  $\beta$ -cell marker as compared to untreated controls.

15 14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10  $\mu$ g/ml

Aprotinin (50 $\mu$ g/ml) 1:2000 (Boehringer manheim #981532)

20 Bovine pituitary extract (BPE) 60 $\mu$ g/ml

Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

Transferrin, 100mg (Sigma T2252)

Epidermal Growth Factor, 100 $\mu$ g (BRL 100004)

25 Triiodothyronine, 10 $\mu$ l of 5x10<sup>-6</sup> M (Sigma T5516)

Ethanolamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma E0135)

Phosphoethalamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma P0503)

Selenium, 4 $\mu$ l of 10<sup>-1</sup> M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

30 Hydrocortisone, 2 $\mu$ l of 5X10<sup>-3</sup> M (Sigma #H0135)

Progesterone, 100 $\mu$ l of 1X10<sup>-3</sup> M (Sigma #P6149)

Forskolin, 500 $\mu$ l of 20mM (Calbiochem #344270)

Minimal media:

35 RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml), aprotinin (50  $\mu$ g/ml) and BPE (15  $\mu$ g/ml).

Defined media:

RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml) and aprotinin (50  $\mu$ g/ml).

The following polypeptides were positive in this assay: PRO1275 and PRO1474.

**EXAMPLE 147: Fetal Hemoglobin Induction in an Erythroblastic Cell Line (Assay 107)**

This assay is useful for screening PRO polypeptides for the ability to induce the switch from adult hemoglobin to fetal hemoglobin in an erythroblastic cell line. Molecules testing positive in this assay are expected to be useful for therapeutically treating various mammalian hemoglobin-associated disorders such as the various thalassemias. The assay is performed as follows. Erythroblastic cells are plated in standard growth medium at 1000 cells/well in a 96 well format. PRO polypeptides are added to the growth medium at a concentration of 0.2% or 2% and the cells are incubated for 5 days at 37°C. As a positive control, cells are treated with 100µM hemin and as a negative control, the cells are untreated. After 5 days, cell lysates are prepared and analyzed for the expression of gamma globin (a fetal marker). A positive in the assay is a gamma globin level at least 2-fold above the negative control.

The following polypeptides tested positive in this assay: PRO1478, PRO1265, PRO1412, PRO1279, PRO1304, PRO1306, PRO1418, PRO1410 and PRO1575.

**EXAMPLE 148: Detection of Polypeptides That Affect Glucose and/or FFA Uptake in Skeletal Muscle (Assay 106)**

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by skeletal muscle cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by skeletal muscle would be beneficial including, for example, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat differentiated skeletal muscle, and allowed to incubate overnight. Then fresh media with the PRO polypeptide and +/- insulin are added to the wells. The sample media is then monitored to determine glucose and FFA uptake by the skeletal muscle cells. The insulin will stimulate glucose and FFA uptake by the skeletal muscle, and insulin in media without the PRO polypeptide is used as a positive control, and a limit for scoring. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as either stimulators or inhibitors of glucose and/or FFA uptake in this assay: PRO1130, PRO1275, PRO1418, PRO1555 and PRO1787.

**EXAMPLE 149: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)**

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO1265, PRO1283, PRO1279, PRO1303, PRO1306, PRO1325, PRO1565 and PRO1567.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay:  
PRO1194, PRO1190, PRO1326, PRO1343, PRO1480, PRO1474, PRO1575 and PRO1760.

#### EXAMPLE 150: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4 µg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100 µl of the same media without serum and 100 µl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 µl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO1265, PRO1250, PRO1430, PRO1356, PRO1275, PRO1274, PRO1286, PRO1273, PRO1283, PRO1279, PRO1306, PRO1325, PRO1343, PRO1418, PRO1565, PRO1474, PRO1787, PRO1556 and PRO1801.

#### EXAMPLE 151: Induction of Pancreatic β-Cell Precursor Proliferation (Assay 117)

This assay shows that certain polypeptides of the invention act to induce an increase in the number of pancreatic β-cell precursor cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either β-cell precursors or mature β-cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is a transcription factor called Pdx1.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20 µg/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are

distributed per well. The culture medium for this primary culture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant  $\beta$ -cell marker as compared to untreated controls.

5 14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10  $\mu$ g/ml

Aprotinin (50 $\mu$ g/ml) 1:2000 (Boehringer manheim #981532)

10 Bovine pituitary extract (BPE) 60 $\mu$ g/ml

Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

Transferrin, 100mg (Sigma T2252)

Epidermal Growth Factor, 100 $\mu$ g (BRL 100004)

15 Triiodothyronine, 10 $\mu$ l of  $5 \times 10^{-6}$  M (Sigma T5516)

Ethanolamine, 100 $\mu$ l of  $10^{-1}$  M (Sigma E0135)

Phosphoethanolamine, 100 $\mu$ l of  $10^{-1}$  M (Sigma P0503)

Selenium, 4 $\mu$ l of  $10^{-1}$  M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

20 Hydrocortisone, 2 $\mu$ l of  $5 \times 10^{-3}$  M (Sigma #H0135)

Progesterone, 100 $\mu$ l of  $1 \times 10^{-3}$  M (Sigma #P6149)

Forskolin, 500 $\mu$ l of 20mM (Calbiochem #344270)

Minimal media:

RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml), aprotinin (50  $\mu$ g/ml)

25 and BPE (15  $\mu$ g/ml).

Defined media:

RPMI 1640 plus transferrin (10  $\mu$ g/ml), insulin (1  $\mu$ g/ml), gentamycin (100 ng/ml) and aprotinin (50  $\mu$ g/ml).

The following polypeptides tested positive in this assay: PRO1382 and PRO1561.

30

#### EXAMPLE 152: Proliferation of Rat Utricular Supporting Cells (Assay 54)

This assay shows that certain polypeptides of the invention act as potent mitogens for inner ear supporting cells which are auditory hair cell progenitors and, therefore, are useful for inducing the regeneration of auditory hair cells and treating hearing loss in mammals. The assay is performed as follows. Rat UEC-4 utricular epithelial cells are aliquoted into 96 well plates with a density of 3000 cells/well in 200  $\mu$ l of serum-containing medium at 33°C. The cells are cultured overnight and are then switched to serum-free medium at 37°C. Various dilutions of PRO polypeptides (or nothing for a control) are then added to the cultures and the cells are incubated for 24 hours. After the 24 hour incubation,  $^3$ H-thymidine (1  $\mu$ Ci/well) is added and the cells

are then cultured for an additional 24 hours. The cultures are then washed to remove unincorporated radiolabel, the cells harvested and Cpm per well determined. Cpm of at least 30% or greater in the PRO polypeptide treated cultures as compared to the control cultures is considered a positive in the assay.

The following polypeptides tested positive in this assay: PRO1340.

#### 5 EXAMPLE 153: Chondrocyte Proliferation Assay (Assay 111)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce the proliferation and/or redifferentiation of chondrocytes in culture. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis.

10 Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4 µg/ml gentamycin. The culture media is changed every third day and the cells are reseeded to 25,000 cells/cm<sup>2</sup> every five days. On day 12, the cells are seeded in 96 well plates at 5,000 cells/well in 100 µl of the same media without serum and 100 µl of either serum-free medium (negative control), staurosporin (final concentration of 5 nM; positive control) or the test PRO polypeptide are added to give a final volume of 200 µl/well. After 5 days at 37°C, 20 µl of Alamar blue is added to each well and the plates are incubated for an additional 3 hours at 37°C. The fluorescence is then measured in each well (Ex: 530 nm; Em: 590 nm). The fluorescence of a plate containing 200 µl of the serum-free medium is measured to obtain the background. A positive result in the assay is obtained when the fluorescence of the PRO polypeptide treated sample is more like that of the positive control than the negative control.

20 The following PRO polypeptides tested positive in this assay: PRO1265, PRO1412, PRO1347, PRO1279, PRO1410 and PRO1474.

#### EXAMPLE 154: Inhibition of Heart Neonatal Hypertrophy Induced by LIF+ET-1 (Assay 74)

25 This assay is designed to determine whether PRO polypeptides of the present invention show the ability to inhibit neonatal heart hypertrophy induced by LIF and endothelin-1 (ET-1). A test compound that provides a positive response in the present assay would be useful for the therapeutic treatment of cardiac insufficiency diseases or disorders characterized or associated with an undesired hypertrophy of the cardiac muscle.

30 Cardiac myocytes from 1-day old Harlan Sprague Dawley rats (180 µl at 7.5 x 10<sup>4</sup>/ml, serum <0.1, freshly isolated) are introduced on day 1 to 96-well plates previously coated with DMEM/F12 + 4%FCS. Test PRO polypeptide samples or growth medium alone (negative control) are then added directly to the wells on day 2 in 20 µl volume. LIF + ET-1 are then added to the wells on day 3. The cells are stained after an additional 2 days in culture and are then scored visually the next day. A positive in the assay occurs when the PRO polypeptide treated myocytes are visually smaller on the average or less numerous than the untreated myocytes.

35 The following PRO polypeptides tested positive in this assay: PRO1760.

#### EXAMPLE 155: Tissue Expression Distribution

Oligonucleotide probes were constructed from some of the PRO polypeptide-encoding nucleotide sequences shown in the accompanying figures for use in quantitative PCR amplification reactions. The

oligonucleotide probes were chosen so as to give an approximately 200-600 base pair amplified fragment from the 3' end of its associated template in a standard PCR reaction. The oligonucleotide probes were employed in standard quantitative PCR amplification reactions with cDNA libraries isolated from different human adult and/or fetal tissue sources and analyzed by agarose gel electrophoresis so as to obtain a quantitative determination of the level of expression of the PRO polypeptide-encoding nucleic acid in the various tissues tested. Knowledge of the expression pattern or the differential expression of the PRO polypeptide-encoding nucleic acid in various different human tissue types provides a diagnostic marker useful for tissue typing, with or without other tissue-specific markers, for determining the primary tissue source of a metastatic tumor, and the like. These assays provided the following results.

	<u>DNA Molecule</u>	<u>Tissues With Significant Expression</u>	<u>Tissues Lacking Significant Expression</u>
10	DNA19902-1669 DNA23322-1393 DNA26846-1397 DNA56107-1415 DNA56406-1704	HUVEC cells, colon tumor uterus, colon tumor, prostate lymphoblast cells spleen, substantia nigra, colon tumor THP-1 macrophages, uterus, spleen	dendritic cells, lymphoblast cells, heart cartilage uterus, heart, cartilage cartilage endothelial cells, prostate, cartilage
15	DNA56529-1647 DNA56862-1343	liver, kidney, brain endothelial cells, substantia nigra hippocampus	adenocarcinoma, lung, bone marrow colon tumor, lymphoblast cells, uterus
	DNA57254-1477 DNA58730-1607	kidney bone marrow, kidney	lung, placenta, brain lung, brain
20	DNA58732-1650 DNA58828-1519 DNA58852-1637 DNA59212-1627	lung, bone marrow adenocarcinoma uterus uterus	brain, liver lung, retina, small intestine colon tumor, heart, brain prostate, cartilage, heart
25	DNA59219-1613 DNA59817-1703 DNA60278-1530 DNA60608-1577 DNA60611-1524 DNA60740-1615	spleen, dendrocytes, prostate, uterus bone marrow prostate, colon tumor kidney, bone marrow breast carcinoma breast carcinoma, adenocarcinoma	substantia nigra, colon tumor, heart lung, small intestine, placenta uterus, cartilage breast carcinoma, small intestine, lung lung, small intestine, retina lung, small intestine, brain
30	DNA62809-1531 DNA62815-1576 DNA62845-1684 DNA64849-1604	THP-1 macrophages colon tumor, uterus, prostate liver, bone marrow kidney	uterus, spleen, brain, colon tumor spleen, brain, heart, cartilage adenocarcinoma, lung, brain lung, pancreas, liver, thyroid
35	DNA64863-1573 DNA64881-1602 DNA64902-1667 DNA64952-1568 DNA65403-1565	lung, brain, kidney, bone marrow uterus uterus lung, brain spleen, dendrocytes, THP-1 macrophages	liver, pancreas heart, spleen, brain, endothelial cells prostate, brain, heart, spleen pancreas endothelial cells, colon tumor, lymphoblasts
40	DNA65408-1578 DNA65423-1595 DNA66512-1564 DNA66519-1535 DNA66521-1583	prostate, spleen, dendrocytes testis heart, uterus, prostate, cartilage dendrocytes, lymphoblasts, uterus uterus, heart, hippocampus	uterus, heart, substantia nigra breast carcinoma, retina, small intestine endothelial cells substantia nigra, prostate, spleen cartilage, dendrocytes, spleen
45	DNA66658-1584 DNA66672-1586 DNA66674-1599	prostate, uterus, hippocampus, spleen spleen uterus, prostate	colon tumor, cartilage, heart heart, prostate, brain, uterus heart, brain, spleen, cartilage, colon tumor
50	DNA68836-1656 DNA68871-1638 DNA68880-1676 DNA68885-1678 DNA71180-1655	kidney uterus, colon tumor, prostate heart, endothelial cells, brain, uterus uterus, colon tumor, prostate brain	lung, brain, bone marrow, liver heart, cartilage, brain, spleen THP-1 macrophages brain, heart, cartilage, endothelial cells lung, bone marrow, liver, kidney

	DNA71184-1634	breast carcinoma, bone marrow, testis	brain, adrenal gland
	DNA71234-1651	kidney, bone marrow	lung, brain, placenta
	DNA71277-1636	prostate, cartilage, heart, uterus	colon tumor, substantia nigra, endothelial cells
	DNA71286-1687	uterus, prostate, brain, cartilage	heart
5	DNA71883-1660	aortic endothelial cells	lung, retina, small intestine, kidney
	DNA73492-1671	breast carcinoma, aortic endothelial cells	lung, brain, testis
		bone marrow	
	DNA73734-1680	prostate, spleen	heart, cartilage, brain, uterus
	DNA73735-1681	prostate	brain, heart, cartilage, spleen
10	DNA73736-1657	spleen, substantia nigra, hippocampus, cartilage	prostate, heart, uterus, dendrocytes
	DNA73737-1658	uterus	
	DNA73742-1662	spleen, uterus, prostate	prostate, heart, spleen, cartilage
			dendrocytes, colon tumor, endothelial cells
15	DNA73746-1654	prostate	uterus, heart, brain, cartilage, spleen
	DNA73760-1672	breast carcinoma	retina, brain, kidney, liver, testis
	DNA76393-1664	endothelial cells, cartilage, uterus	brain, prostate
	DNA76398-1699	hippocampus, prostate, THP-1 macrophages	heart, uterus, spleen, dendrocytes
20	DNA76399-1700	IM-9 lymphoblasts	
	DNA76522-2500	colon tumor	prostate, spleen, heart, cartilage, uterus
	DNA77301-1708	brain	uterus, prostate, brain, heart, cartilage
	DNA77648-1688	retina, breast carcinoma, kidney, liver, bone marrow	lung, small intestine, kidney, liver
			brain, lung
25	DNA77568-1626	brain	
	DNA58727-1474	HUVEC, dendrocytes, uterus	lung, liver, placenta, heart
			substantia nigra, hippocampus, prostate, colon tumor
	DNA61185-1646	colon tumor, HUVEC	uterus, dendrocytes, substantia nigra
	DNA61608-1606	colon tumor, dendrocytes, spleen, testis	substantia nigra, placenta
30	DNA66304-1546	prostate, testis	uterus, brain, heart, colon tumor, adrenal gland
	DNA71213-1659	brain, spleen, HUVEC, colon tumor	prostate, uterus, heart, cartilage
	DNA62812-1594	heart	placenta, testis, uterus, adrenal gland, bone marrow, prostate
			bone marrow
35	DNA66660-1585	colon tumor, HUVEC, testis, placenta, uterus	
	DNA66669-1597	heart, placenta, adrenal gland, uterus	cartilage, testis, colon tumor, HUVEC, bone marrow, prostate, spleen
	DNA68866-1644	testis, colon tumor, prostate, spleen,	cartilage, adrenal gland, HUVEC, placenta
40	DNA73730-1679	testis, adrenal gland, uterus, prostate, uterus	cartilage, colon tumor, heart, placenta, spleen



### Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

Table 12

	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
5	DNA19902-1669	203454	November 3, 1998
	DNA26846-1397	203406	October 27, 1998
	DNA56107-1415	203405	October 27, 1998
	DNA56406-1704	203478	November 17, 1998
	DNA56529-1647	203293	September 29, 1998
10	DNA56531-1648	203286	September 29, 1998
	DNA56862-1343	203174	September 1, 1998
	DNA57254-1477	203289	September 29, 1998
	DNA57841-1522	203458	November 3, 1998
	DNA58727-1474	203171	September 1, 1998
15	DNA58730-1607	203221	September 15, 1998
	DNA58732-1650	203290	September 29, 1998
	DNA58828-1519	203172	September 1, 1998
	DNA58852-1637	203271	September 22, 1998
	DNA59212-1627	203245	September 9, 1998
20	DNA59218-1559	203287	September 29, 1998
	DNA59219-1613	203220	September 15, 1998
	DNA59586-1520	203288	September 29, 1998
	DNA59817-1703	203470	November 17, 1998
	DNA60278-1530	203170	September 1, 1998
25	DNA60608-1577	203126	August 18, 1998
	DNA60611-1524	203175	September 1, 1998
	DNA60618-1557	203292	September 29, 1998
	DNA60740-1615	203456	November 3, 1998
	DNA60764-1533	203452	November 10, 1998
30	DNA60775-1532	203173	September 1, 1998
	DNA61185-1646	203464	November 17, 1998
	DNA61608-1606	203239	September 9, 1998
	DNA62808-1326	203358	October 20, 1998
	DNA62809-1531	203237	September 9, 1998
35	DNA62815-1578	203247	September 9, 1998
	DNA62845-1684	203361	October 20, 1998
	DNA64842-1632	203278	September 22, 1998
	DNA64849-1604	203468	November 17, 1998
	DNA64863-1573	203251	September 9, 1998
40	DNA64881-1602	203240	September 9, 1998
	DNA64883-1526	203253	September 9, 1998
	DNA64885-1529	203457	November 3, 1998
	DNA64886-1601	203241	September 9, 1998
	DNA64888-1542	203249	September 9, 1998
45	DNA64889-1541	203250	September 9, 1998
	DNA64897-1628	203216	September 15, 1998
	DNA64902-1667	203317	October 6, 1998
	DNA64903-1553	203223	September 15, 1998
	DNA64905-1558	203233	September 15, 1998
50	DNA64950-1590	203224	September 15, 1998
	DNA64952-1568	203222	September 15, 1998
	DNA65402-1540	203252	September 9, 1998
	DNA65403-1565	203230	September 15, 1998
	DNA65404-1551	203244	September 9, 1998
55	DNA65405-1547	203476	November 17, 1998
	DNA65406-1567	203219	September 15, 1998

	DNA65408-1578	203217	September 15, 1998
	DNA65409-1566	203232	September 15, 1998
	DNA65410-1569	203231	September 15, 1998
	DNA65423-1595	203227	September 15, 1998
	DNA66304-1546	203321	October 6, 1998
5	DNA66511-1411	203228	September 15, 1998
	DNA66512-1564	203218	September 15, 1998
	DNA66519-1535	203236	September 15, 1998
	DNA66520-1536	203226	September 15, 1998
	DNA66521-1583	203225	September 15, 1998
10	DNA66526-1616	203246	September 9, 1998
	DNA66658-1584	203229	September 15, 1998
	DNA66659-1593	203269	September 22, 1998
	DNA66663-1598	203268	September 22, 1998
	DNA66669-1597	203272	September 22, 1998
15	DNA66672-1586	203265	September 22, 1998
	DNA66674-1599	203281	September 22, 1998
	DNA66675-1587	203282	September 22, 1998
	DNA67962-1649	203291	September 29, 1998
	DNA68836-1656	203455	November 3, 1998
20	DNA68864-1629	203276	September 22, 1998
	DNA68866-1644	203283	September 22, 1998
	DNA68871-1638	203280	September 22, 1998
	DNA68874-1622	203277	September 22, 1998
	DNA68880-1676	203319	October 6, 1998
25	DNA68885-1570	203311	October 6, 1998
	DNA71166-1685	203355	October 20, 1998
	DNA71169-1709	203467	November 17, 1998
	DNA71180-1655	203403	October 27, 1998
	DNA71184-1634	203266	September 22, 1998
30	DNA71213-1659	203401	October 27, 1998
	DNA71234-1651	203402	October 27, 1998
	DNA71277-1636	203285	September 22, 1998
	DNA71282-1668	203312	October 6, 1998
	DNA71286-1604	203357	October 20, 1998
35	DNA71883-1660	203475	November 17, 1998
	DNA73401-1633	203273	September 22, 1998
	DNA73492-1671	203324	October 6, 1998
	DNA73727-1673	203459	November 3, 1998
	DNA73730-1679	203320	October 6, 1998
40	DNA73734-1680	203363	October 20, 1998
	DNA73735-1681	203356	October 20, 1998
	DNA73736-1657	203466	November 17, 1998
	DNA73737-1658	203412	October 27, 1998
	DNA73739-1645	203270	September 22, 1998
45	DNA73742-1662	203316	October 6, 1998
	DNA73744-1665	203322	October 6, 1998
	DNA73746-1654	203411	October 27, 1998
	DNA73760-1672	203314	October 6, 1998
	DNA76396-1698	203471	November 17, 1998
50	DNA76398-1699	203474	November 17, 1998
	DNA76399-1700	203472	November 17, 1998
	DNA76401-1683	203360	October 20, 1998
	DNA76510-2504	203477	November 17, 1998
	DNA76522-2500	203469	November 17, 1998
55	DNA76529-1666	203315	October 6, 1998
	DNA76531-1701	203465	November 17, 1998
	DNA76532-1702	203473	November 17, 1998
	DNA76538-1670	203313	October 6, 1998

	DNA76541-1675	203409	October 27, 1998
	DNA77301-1708	203407	October 27, 1998
	DNA77303-2502	203479	November 17, 1998
	DNA77648-1688	203408	October 27, 1998
	DNA77652-2505	203480	November 17, 1998
5	DNA83500-2506	203391	October 29, 1998
	DNA77568-1626	203134	August 18, 1998
	DNA23322-1393	203400	October 27, 1998
	DNA59814-1486	203359	October 20, 1998
	DNA62812-1594	203248	September 9, 1998
10	DNA66660-1585	203279	September 22, 1998
	DNA76393-1664	203323	October 6, 1998

These deposit were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.